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CRYSTALLINE AXES OF THE SPINE AND TEST OF THE SEA URCHIN *STRONGYLOCENTROTUS PURPURATUS*: DETERMINATION BY CRYSTAL ETCHING AND DECORATION

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Abstract

By means of crystal etching and scanning electron microscopy the c- and a-axes of skeletal units of *Strongylocentrotus purpuratus* have been determined. Crystal etching and crystal decoration were found to be equally useful in demonstrating that the spines are single crystals of magnesian calcite. All regions of spine cross sections had common c- and a-axes. Comparisons of etched figures to decorated crystals on the same cross section of a spine showed that the grooves of the etch figures were parallel to the edges of the upper faces of the decorated crystals. The a-axes of the primary spines differed from those of the interambulacral plates to which they were attached.

Certain regions of the tubercle were shown by SEM examination of decorated tests to be polycrystalline aggregates lacking uniform crystal orientation, whereas the remaining portions of the interambulacral plates shared the same a-axes. The primary plates and demiplates of the ambulacral plates were each single crystals whose a-axis, and often c-axis, orientations were independent of adjacent compartments.

Reflective properties of decorated plates coated with gold-palladium have been shown to be useful in demonstrating differences in crystal orientation between adjacent interambulacral plates and between compartments of individual ambulacral plates.

Introduction

The skeleton of the Echinoidea is of crystallographic interest because of its ultrastructure and the orientation of its units. The spines and plates are magnesian calcite formed as a mineral meshwork of smooth trabeculae by cells of the dermis within the interconnecting spaces (Weber *et al.*, 1969; Heatfield, 1971; Märkel, 1981). X-ray diffraction and polarized light studies have indicated that the spines and plates, with the exception of tubercles which bear spines, are single crystals (Schmidt, 1930, 1932; West, 1937; Donnay, 1956; Raup, 1962, 1966; Currey and Nichols, 1967; Donnay and Pawson, 1969; Nissen, 1969).

By means of polarization microscopy, it has been shown that in spines the c-axis is parallel to the axis of elongation (Schmidt, 1930, 1932; West, 1937; Raup, 1962, 1966) and that in the ambulacral and interambulacral plates of *Strongylocentrotus purpuratus* the c-axis is normal to the outer surface, an orientation common to many, but not all, species (Raup, 1962). Other crystal axes were not determined in these studies since the optical method is limited to defining the

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direction of the c-axis. X-ray diffraction, which can resolve the c-axis and the a-axes, has been applied to the analysis of echinoid skeletons in only a limited way and has been directed primarily to the question as to whether the spines and plates constitute single crystals (Garrido and Blanco, 1947; Donnay, 1956; Donnay and Pawson, 1969).

The method of crystal decoration has proved valuable in the crystallographic analysis of larval and adult echinoid skeletons (Okazaki and Inoué, 1976; Okazaki *et al.*, 1980; Dillaman and Hart, 1981) as well as calcareous sponge spicules (Jones, 1955) and the test of a foraminiferan (Towe, 1977). In this method, skeletal structures placed in a saturated solution of $CaCO_3$ become decorated with calcite crystals whose orientation indicates the orientation of the crystals on which they form. Observations of the decorated crystals by electron microscopy permits determination of the c- and a-axes (Dillaman and Hart, 1981).

The view that the plates of the echinoderm test are single crystals has not found universal acceptance. Based on evidence from polarized light and observation of surface replicas by transmission electron microscopy, Towe (1967) suggested that interambulacral plates, which appear to be single crystals, are formed by oriented polycrystalline growth followed by a maturation involving recrystallization and continuous fusion and coalescence. The evidence presented does not include information on the a-axes of the skeletal elements and for this reason cannot completely define crystal orientation. By means of Laue X-ray diffraction and crystal decoration, Dillaman and Hart (1981) were able to show that the interambulacral plates conform to the criteria of single crystals as indicated by c- and a-axis orientation. Further, examination of the ultrastructure of the fenestrated plates by scanning electron microscopy gave no evidence of discontinuities or a polycrystalline structure.

In the present study we have carried out further detailed analysis of the skeletal structure of *Strongylocentrotus purpuratus* by the method of crystal etching. It appeared possible that by producing etch figures the crystal planes of skeletal units might be made evident and so permit the determination of c- and a-axes and crystal orientation. This has proved to be the case. Results obtained by crystal etching were found to give results equivalent to those by crystal decoration. Then, using decoration, we compared crystal orientation of plates and attached spines and the orientation of the crystal compartments within single ambulacral plates of the test.

Materials and Methods

Specimens of adult *Strongylocentrotus purpuratus* obtained from Pacific Biomarine Laboratories, Venice, California were held in large recirculating sea water tanks at room temperature. Samples of primary spines, ambulacral and interambulacral plates were prepared by removing Aristotle's lantern and internal organs from living urchins and immersing the remaining skeletal material and attached soft tissue in Clorox (5.25% sodium hypochlorite) for 5 hours. This treatment removed all organic material covering and connecting the skeletal components. After 5 washes with distilled water adjusted to pH 8.0 with NaOH, primary spines and coronal plates were dehydrated through 70%, 95% and 100% ethanol and stored in 100% ethanol.

Etching of spines and plates

For etching of skeletal elements, Clorox-treated spines and plates were returned to distilled water, pH 8.0, by hydration through 95% and 70% ethanol. Entire

spines and those fractured at right angles to the long axis were then immersed in 1–3% acetic acid (pH 2.6–2.8) for 4–10 minutes. Coronal plates were disarticulated and treated with 0.1–0.01% acetic acid (pH 3.2–3.7) for 10 minutes. Etching was stopped by transfer to 0.01 *N* NaOH, followed by repeated washes in distilled water, pH 8.0.

Decoration of spines and plates

Etched spines and plates were decorated with calcite crystals following the method of Okazaki *et al.* (1980). Samples in distilled water were transferred into a 0.1 *M* $NaHCO_3$ solution to which was added a 0.1 *M* $CaCl_2$ solution to give a ratio of the two solutions of 5:2. After 5 minutes in the final mixture, specimens were washed with distilled water, pH 8.0, and dehydrated through an ascending series of ethanol.

Scanning electron microscopy

Untreated, etched, and etched and decorated spines and plates after dehydration with ethanol were air-dried and attached to aluminum stubs with Duco cement. After sputter-coating with gold-palladium, specimens were examined with a Philips 501 scanning electron microscope at 15 or 30 KV.

Results

1. Structure of untreated coronal plates and spines.

Coronal plates. The corona consists of 20 curved rows of plates, five ambulacral areas of two-plate rows alternating with five interambulacral two-plate rows (Fig. 1A). Each plate bears one large primary tubercle on which the primary spine is situated. There are several secondary tubercles with spines surrounding the primary tubercle (Figs. 1D, 6A, B). All tubercles consist of a basal portion (the boss) having the shape of a low truncated cone which is surmounted by a terminal knob (the mamelon) lying on a narrow shoulder. The boss is encircled by a bare area to which are attached the muscles operating the spine. These four parts of the tubercle are designated as regions 1, 2, 3, and 4 (Fig. 6C).

Each ambulacral plate is an assembly of seven compartments, each pierced by seven paired pores for the passage of the podia (Hyman, 1955). These compartments consist of two primary plates (Fig. 6A, a, g) and five demiplates inserted between them (Fig. 6A, b, c, d, e, f). Every part of the coronal plates exhibits a fenestrated structure with the exception of the outer surface of the mamelon, which is solid (Figs. 6A, B, and C). Under the scanning electron microscope, surfaces of the coronal plates and spines appear smooth and do not display any surface texture suggestive of their crystalline composition, as pointed out by Currey and Nichols (1967) and Dillaman and Hart (1981).

Primary spines. The spines of *Strongylocentrotus purpuratus* have been described in detail by Heatfield (1971). The main regions are a basal portion, a milled ring, and a tapering shaft (Fig. 1B, C). The concave surface of the base and the mamelon of the tubercle of the associate plate form a ball-and-socket joint (Fig. 1C).

In transverse section, the spine is characterized by a series of calcite rings (Fig. 2A) in a continuous meshwork. In the outer zone, wedges of solid calcite alternate with the meshwork to form radii and concentric rings. The outermost surfaces of

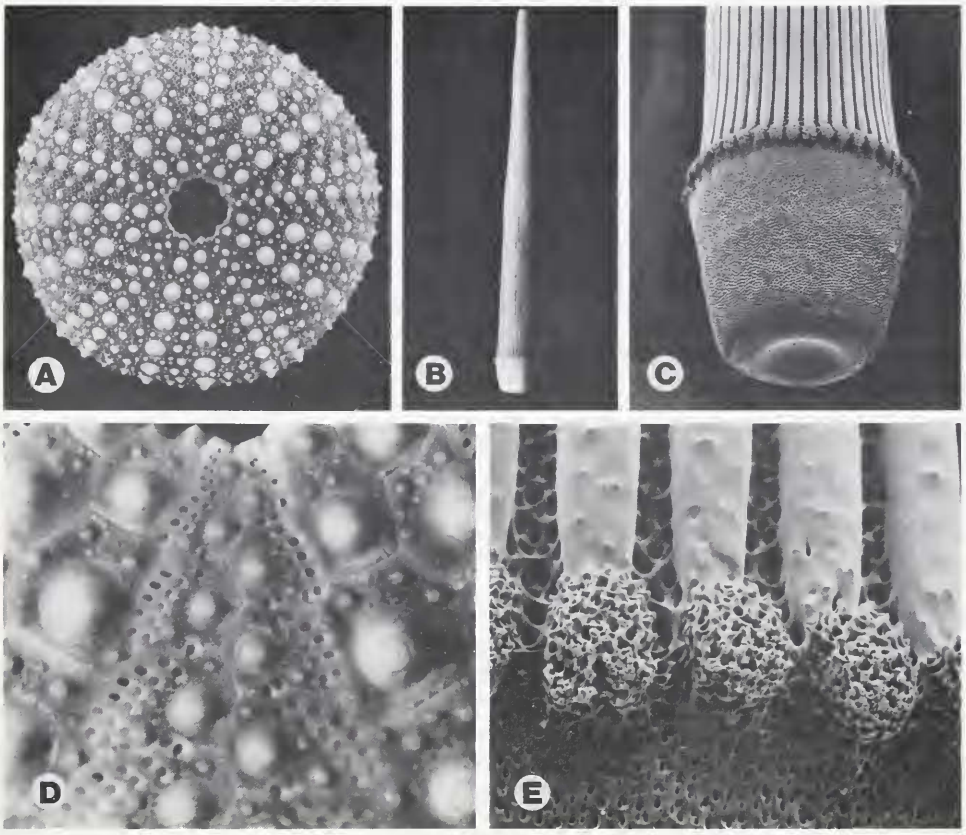

FIGURE 1. A. Light micrograph of Clorox-treated test showing curved rows of plates, ×2. B. Light micrograph of spine showing the basal portion, milled ring and tapered shaft, ×5. C. Scanning electron micrograph of spine showing concave base, ×25. D. Light micrograph of test showing double row of ambulacral plates, each pierced by seven pairs of pores. Also apparent are the mamelons that accept the spine, ×10. E. Scanning electron micrograph of base of spine showing trabecular components between the longitudinal columns, ×210.

the wedges are smooth and rounded and appear as longitudinal columns in side view (Fig. 1C, E). The number of columns on a single mature spine ranged from 40 to 60, and the number frequently increased from the milled ring to the tip of a spine. In transverse section, the increase was seen as new radii (Fig. 2A). In the present study, the wedges forming the columns were loci for observations of etch figures and decorated crystals.

2. Etch figures and decorated crystals on the spine.

Etch figures created by treating spines with dilute acid are shown in Figures 2B, C, D. Figure 2B is a lateral view showing identical cleavage directions on the two columns when viewed from the same perspective. Figures 2C, D show etch figures of two wedges on a cross section of a spine located in regions I and II, respectively, of Figure 2A. On each wedge, three cleavage faces are evident. The faces are parallel in the two wedges even though they occupy different positions

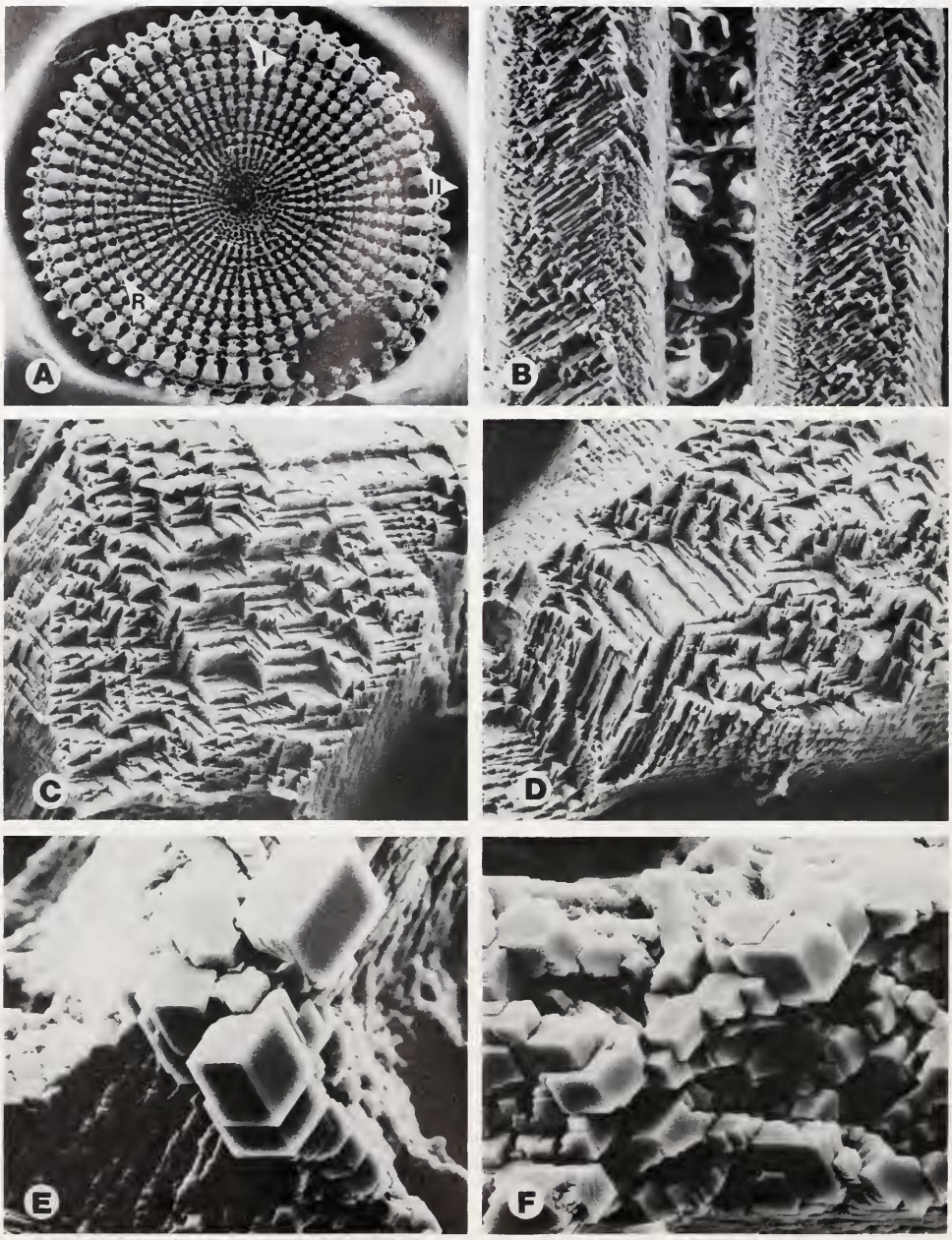

FIGURE 2. Scanning electron micrographs of spines. A. Transverse section of spine showing series of rings. Also note new radius (R) that serves to increase the circumference, ×41. B. Longitudinal column after etching with acetic acid. Note identical cleavage directions in adjacent columns, ×350. C. and D. Etch patterns on surface of transverse section from regions I and II of Fig. 2A. Note that cleavage angles are identical, ×1250. E. and F. Decorated crystals on surface of transverse section at sites comparable to I and II in Fig. 2A. Note that crystal edges are parallel, ×2500.

on the circumference of the spine. Each wedge of the spine shows the same orientation. Since each of the three angles formed by the intersection of the three cleavage faces were 120° it was judged that the observations were made parallel to the electron beam along the c-axis of the spine. One can accordingly conclude from the correspondence of the angles that the spine is a single crystal whose c-axis is normal to the cross-section, that is, parallel to the axis of elongation of the spine. Further, since the angle between the a-axis and the ridge of calcite is 30° when viewed along the c-axis (Fig. 3), etch figures can also be used to determine the a-axes. From etch figures in Figures 2C, D, the three a-axes of Figure 2A were determined and are indicated by the superimposed dark lines of Figure 3.

Because the etch figures were relatively small, it was necessary to make many measurements from a single micrograph for exact determinations of cleavage surfaces. By decorating the etched surfaces, determinations of c- and a-axes were greatly facilitated. The rhombohedral decorated calcite crystals were of sufficient size and the coigns prominent to the extent that the stage of the electron microscope could be oriented so that the ridges of single crystals formed 120° angles with one another. Thus, one could assume that the crystal was being observed along its c-axis. The a-axes could then be calculated. Figures 2E, F are representative of the habits of the crystals as observed at all locations on the cross section of a single spine, including its base. This result supports the conclusion based on etch figures that the spine is a single crystal. Although calcite crystals could be grown on unetched surfaces of the spine, crystals of varying orientation frequently developed, presumably from small chips produced by cutting which served as nuclei.

To test the relationship between etch figures and decorated crystals, individual spines were first etched and half of the spine then decorated, permitting a comparison of etch and decorated figures on the same spine (Fig. 4A, B). An examination of these figures shows that the grooves of the etch figures precisely paralleled the ridges of the decorated crystals. However, as Figure 3 indicates, the ridges on

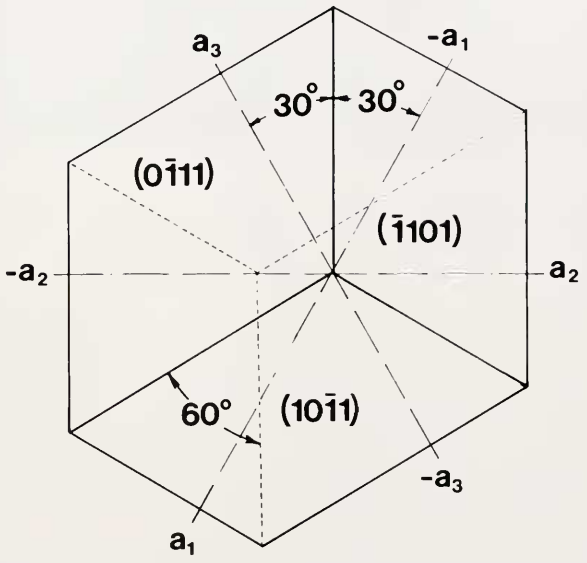

FIGURE 3. Schematic representation of calcite rhombohedron viewed along c-axis. Bold lines represent the upper edges of the rhomb while dotted lines indicate lower edges.

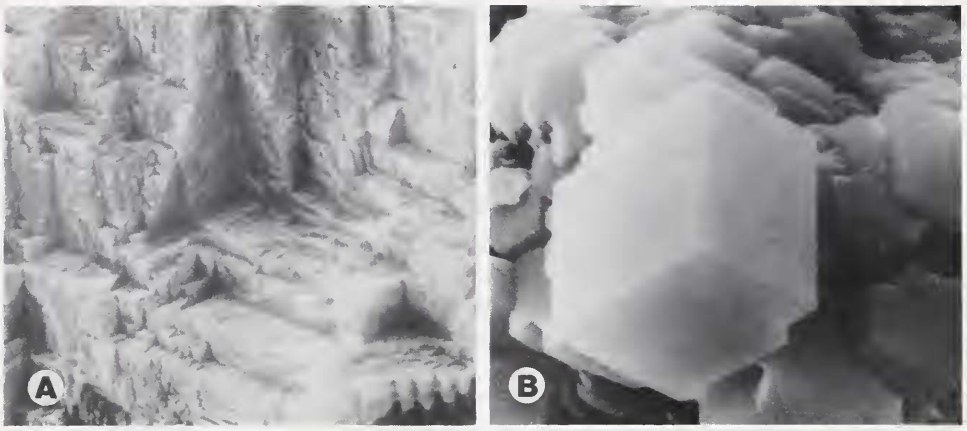

FIGURE 4. Transverse section of a single spine viewed with the scanning electron microscope after etching (A) and after etching and decoration (B). Note that grooves of etch figures parallel the edges of the decorated crystals. A., ×11,000, B., ×5000.

the front form angles of 60° with the ridges on the rear of the calcite rhombohedron when viewed along the c-axis (*cf.* solid lines and dotted lines). Since one would initially assume that the etch figure would represent the removal of calcite along cleavage faces, it was therefore necessary to provide an alternative explanation for the relationship between the etch and decorated figures. As an approach to a possible explanation, mineral calcite whose initial crystal habit was evident was treated in exactly the same manner as the spine and then examined with the scanning electron microscope. When the specimen was observed along its c-axis as determined by its preexisting cleavage plane (Fig. 5A), two types of etch figures were observed, as shown diagrammatically in Figures 5B and C. Scanning electron micrographs of actual pits are shown in Figures 5E and F. Figures 5B and E correspond to a depression due to the removal of a rhombohedral sector, which was characterized by sharp grooves and smooth etch pit walls. Equally frequent were etch figures shown in Figures 5C and F with grooves that paralleled the ridges of the underlying crystal (Fig. 5A). The two lateral walls showed a stepped appearance (Fig. 5F) whereas the third wall was smooth. As a consequence, the groove formed where the two stepped walls met was not as sharp as in the previously mentioned type of etch figure. In both types, however, the grooves formed 120° angles with one another when viewed along the c-axis, demonstrating they were equally capable of revealing the c-axis of the crystal. Irrespective of underlying etch figures, orientations of the edges of the decorated crystals were all the same as the initial calcite (Fig. 5D).

3. Decorated interambulacral and ambulacral plates.

Interambulacral plates. Etched and decorated interambulacral plates (Fig. 6B) examined by scanning electron microscopy showed several patterns of the decorated crystals. On the tip of the tubercle (Fig. 6C, region 1) the crystals were arranged in sectors of varying orientation (Fig. 6E). The individual sectors varied in both their c- and a-axes and consequently represented coarse polycrystalline aggregates. Region 2 of the tubercle (Fig. 6C) was also made up of polycrystalline aggregates. This region of the skeleton was less dense than region 1 due to its more open

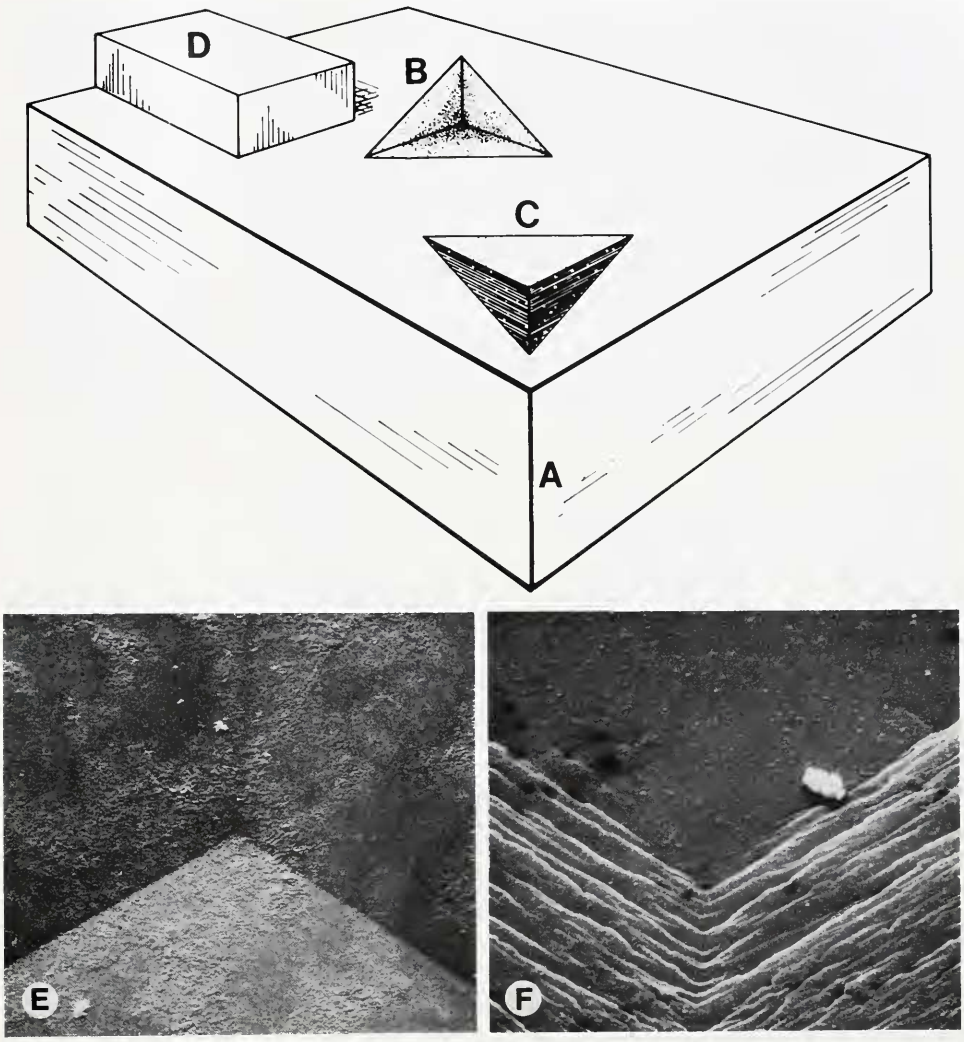

FIGURE 5. Schematic representation of mineral calcite etched and decorated. Cleavage face of native crystal (A) and decorated crystal (D) can be compared with etch pits with smooth sides (B) and stepped sides (C). E. and F. are scanning electron micrographs of etch pits such as indicated in B. and C., respectively. E., ×1900, F., ×2750.

trabecular structure (Fig. 6F). Crystal sectors in region 2 also showed varying c- and a-axes. In regions 3 and 4 of the tubercle (Fig. 6C) and remaining parts of the plate, the decorated crystals were especially large at the growing tips of the trabeculae, and had ridges which were clearly parallel (Fig. 6D). Orientation of the sample so as to form 120° with all the ridges made it possible to determine that the c-axis was approximately perpendicular to the surface of the plate and further permitted calculation of the a-axes. The relationship between the plate margins (exclusive of the margin with the ambulacral plates) was examined. The difference in angle between the a-axes and the sides ranged from 5.9° to 9.5° with a mean value for 24 observations of 8.1° ± 5.0°. The a-axis directions were found

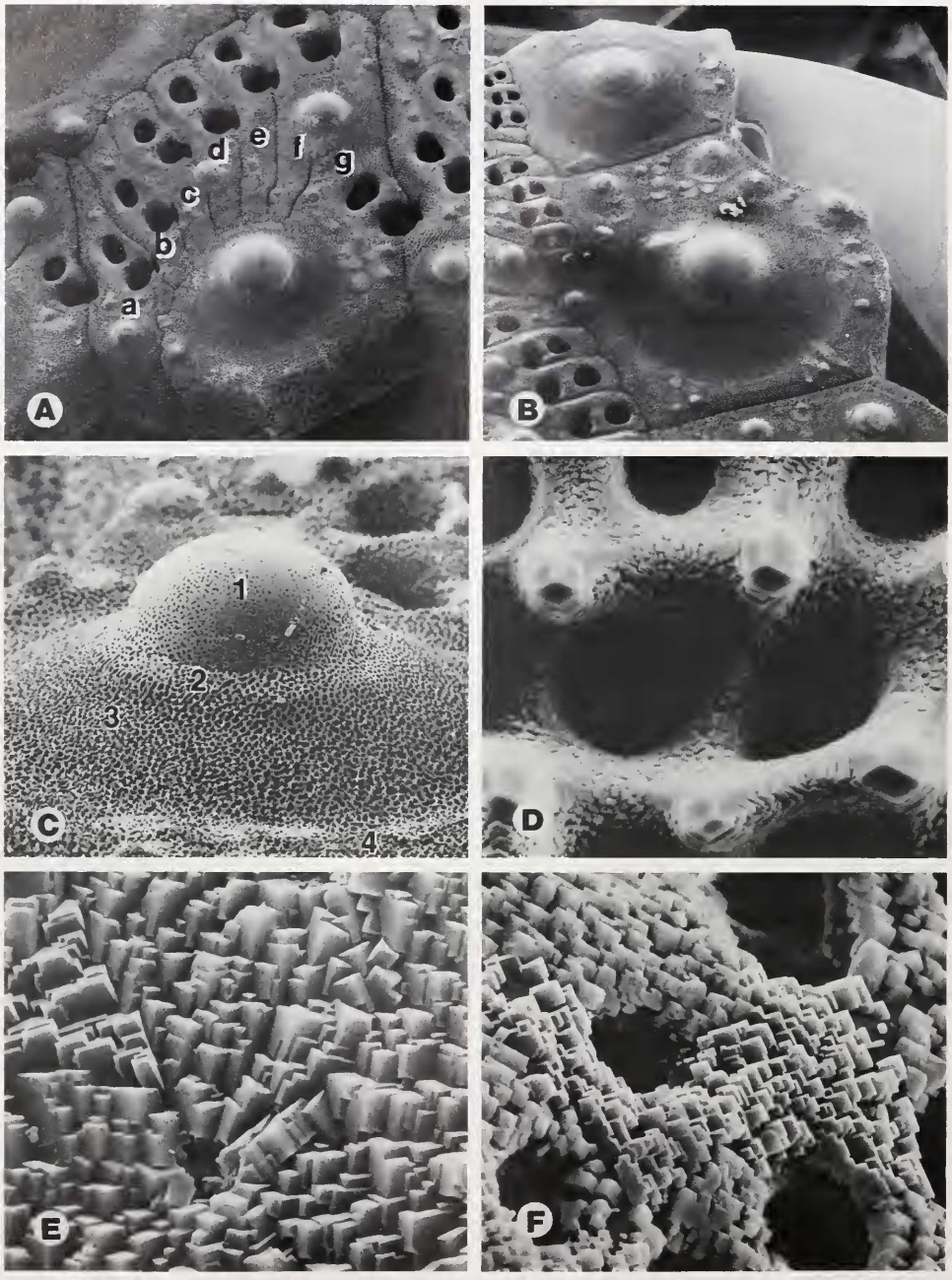

FIGURE 6. Scanning electron micrographs of plates of the test. A. Ambulacral plate showing two primary plates (a and g) and five demiplates (b through f), ×40. B. Interambulacral plates showing location of primary tubercle, ×20. C. Primary tubercle showing terminal knob (1), boss (2 and 3), and underlying plate (4), ×100. D. Decorated interambulacral plate showing large crystal at the growing tips of the trabeculae typical of regions 3 and 4 of Fig. 6C, ×1250. E. Decorated crystals in region 1 of Fig. 6C. Note varying orientation of crystal edges, ×2500. F. Decorated crystals from region 2 of Fig. 6C., ×2000.

to vary in relation to plate margins by Donnay and Pawson (1969) by X-ray diffraction and by Dillaman and Hart (1981) by crystal decoration.

By marking the edge of a spine and tubercle while they were connected and then decorating both, it was possible to compare the a-axes of an interambulacral plate to its primary spine. Figures 7A, B are representative of such preparations. The dark lines indicate their common side. Figures 7C, D correspond to decorated crystals on the plate and spine, respectively. In five such preparations, none indicated that the plate and spine shared the same a-axes.

Ambulacral plates. Scanning electron microscopic examination of etched and decorated ambulacral plates (Fig. 6A) indicated that often the c- and a-axes were different among the seven compartments of a single plate. Measurements of the angles formed by the ridges of individual compartments of two ambulacral plates are given in Table I. Letters a through g refer to compartments as labelled in Figure 6A. In both plates, compartment e was chosen as reference. That is, each plate was rotated so that compartment e was viewed along its c-axis, and then the

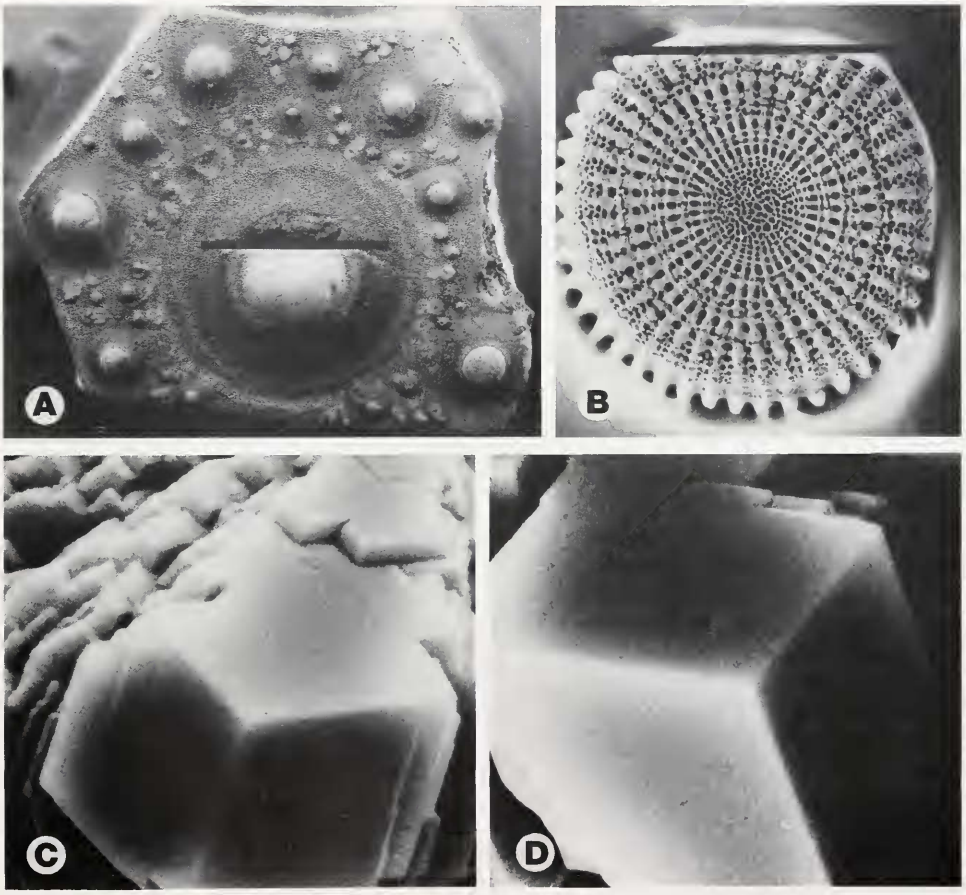

FIGURE 7. Scanning electron micrographs of the tubercle of interambulacral plate (A) and a transverse section of its primary spine (B), each having been flattened on one side to indicate common alignment (Bars). C. and D. are decorated crystals from areas shown in A. and B., respectively. A., ×22, B., ×66, C., and D., ×11,000.

TABLE I

Angles of three cleavage faces surrounding c-axis

Sample No.	Symbol of compartment in ambulacral plate (see Fig. 6 A)						
	a	b	c	d	e	f	g
1	180°	118°	120°	123°	120°	126°	129°
	87	129	124	122	120	118	116
	93	113	116	115	120	116	115
2	120	120	127	120	120	120	120
	120	120	129	120	120	120	120
	120	120	104	120	120	120	120
Shift of a-axis from e	+13	+20	−*	+2	0	−40	−21

*A shift of the a-axis for compartment c could not be measured in that the three angles of reference were not 120°. +, clockwise. −, counterclockwise.

adjacent compartments were viewed with only X and Y translation of the sample. In sample 1 of Table I, no other compartments shared the same c-axis with compartment e, and their deviation was quite varied. In sample 2, six of the seven compartments shared the same c-axis, compartment c being the only one showing a difference. However, when the a-axes of the six compartments of this sample were calculated, it was found that no two had the same orientation. The a-axes ranged from −40° to +20° relative to that of compartment e (Table I, bottom line). Clearly, each compartment of the ambulacral plate was a single crystal with crystal orientation (c- and a-axes) independent of its adjacent compartment.

4. Light reflective properties of decorated and coated skeletal elements.

The thin coat of gold-palladium on the surfaces of decorated spines and coronal plates serving as a conductive layer for scanning electron microscopy effectively made front-surface mirrors of the outer facets (0111, 1101, and 1011) of the calcite crystals. The result was that parallel facets within a given region acted as a single reflecting surface. Figure 8A shows the upper half of a test which was etched, decorated, coated with gold-palladium and illuminated with two flood lamps approximately 45° to the test. Certain interambulacral plates appear very bright while others are dark. The individual areas of ambulacral plates consisting of two primary and five demiplates also exhibited differences in their reflective properties. In Figure 8B, center, two rows of ambulacral plates are shown. In the left row, the top three plates do not reflect, and, in the last one, a single demiplate is bright. In the right row, a variety of reflective patterns is seen. The primary plates, second from top, are bright, and the demiplates are dark. The remaining two plates in the row have a mixed pattern with individual compartments varying from very bright through an intermediate series of grays to dark. Clearly, these results demonstrate that both the interambulacral and ambulacral plates may differ from adjoining plates in their crystal orientation. Within individual ambulacral plates, differences in orientation between compartments were also evident, as analysis of decorated crystals had shown.

DISCUSSION

The methods of etching and crystal decoration coupled with scanning electron microscopy used in the present study have provided information about underlying

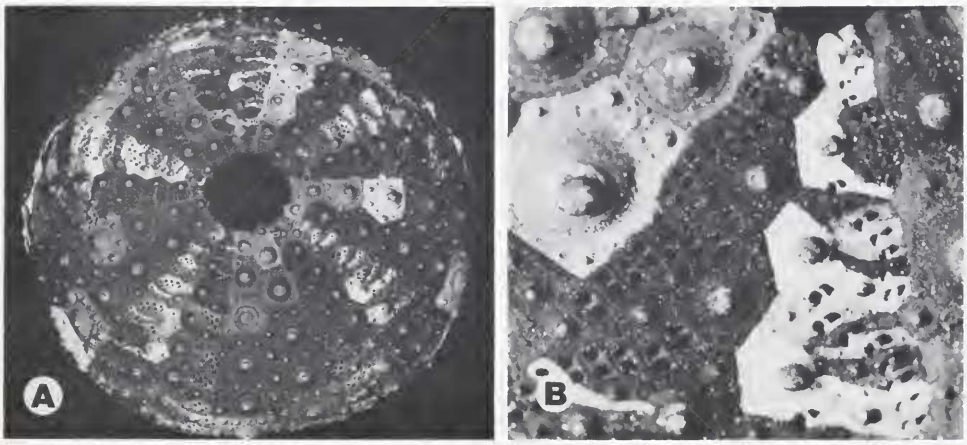

FIGURE 8. Light reflective patterns of test decorated and then coated with gold-palladium. A., ×3, B., ×15.

crystal faces and their relation to the macroscopic and ultrastructure of skeletal units of *Strongylocentrotus purpuratus*. Decoration of etched spines followed by examination with scanning electron microscopy facilitated the determination of a-axes in that the angles of the coigns of the decorated crystals were distinct and more easily measured than etch figures. In general, however, results from etching must be given more weight in that they represent the native crystal faces rather than a derivative. Etching and crystal decoration both showed the spines to be single crystals in that all regions of their cross sections and fine structure had common c- and a-axes.

When etch figures and decorated crystals were compared on the same spine, the grooves of the etch figures and the crystal ridges were parallel. Further, identical treatment of large crystals of mineral calcite showed that decorated crystals reliably reflect c- and a-axes. Etching of these crystals without subsequent decorations gave two types of etch figures, one which could be attributed to removal of unit cells and another parallel to the crystal margins and exhibiting stepping seen in "hopper crystals" (Buerger, 1970). One explanation of "hopper crystals" proposes that the stepping results from a change in the chemical environment of the crystal as it grows (Buerger, 1970). Within the internal environment of the spines, the magnesium and organic material, including pigment, could conceivably change as a result of the activity of the mineralizing cells and so give local changes within the crystal structure. With preferential etching of loci differing in lattice structure, such a stepped figure might be formed. Whatever the reason, it is of interest that mineral calcite with its lower magnesium content and absence of organic material formed two types of etch figures whereas only the stepped pattern was formed in the spines. Regardless of the type of etch figure encountered, the observation that decorated crystals reflect the same c-axis and a-axes as the underlying crystal as shown by etching indicates that decoration is a useful method for determining crystal orientation of sea urchin skeletal parts.

By etching with dilute acetic acid, it has been possible to confirm the polarized light microscopic observations of Raup (1959) showing that the primary spines of *Strongylocentrotus purpuratus* have their c-axis parallel to the axis of elongation. Since the a-axes can be determined from the three grooves of the etch figures, we

could also confirm the conclusion of Donnay and Pawson (1969) from X-ray diffraction that the spine is a single crystal of calcite with a single c-axis and three a-axes.

We found that the a-axes of interambulacral plates and their primary spines did not correspond. One explanation is that the two structures develop independently with respect to their mineralization and with control limited to their c-axes which have the same orientation. A second explanation is that the base of the spine in rotating on the tubercle tip has a grinding action which breaks off small bits of the skeletal material (Donnay and Pawson, 1969). During growth, these bits might fuse to a polycrystalline structure as observed by Towe (1967) and in the present study (Fig. 6E, F).

Our limited analysis of ambulacral plate compartments showed wide diversity of crystal orientation favoring the view that each compartment arises separately. Of 14 plates examined (Table I), no two shared the same a-axes. This is noteworthy in light of the observation that in one plate six of seven compartments had the same c-axis. Analyses restricted to the c-axes would have indicated greater uniformity of crystal orientation than actually existed.

Diversity of orientation among ambulacral plates and compartments of single plates was strikingly evident in whole tests that had been etched, decorated, coated with gold-palladium, and then observed with directed visible light (Fig. 8 A, B). Although the small mean size of the crystal faces caused interference between adjacent reflective surfaces when viewed with a small diameter beam of visible light, this treatment was useful in making general assessments of crystal orientations. Differences that became evident by this simple method could then be analyzed precisely for c- and a-axis orientation using scanning electron microscopy.

Acknowledgments

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